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SOLANUM BULBOCASTANUM LATE BLIGHT
RESISTANCE GENE AND USE THEREOF

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/407,100, filed August 29, 2002. The disclosure of said provisional application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The present invention is directed to pathogen resistance in plants. More particularly, the invention is directed to identification and use of a gene that provides resistance to late blight disease. Even more particularly, the invention is directed to a *Solanum bulbocastanum* late blight resistance gene, nucleic acid molecules encoding polypeptides which confer resistance to late blight, and methods of using the gene, including expression in plant cells to confer or enhance a plant's resistance to late blight.

2. Description of the Art

[0003] On a worldwide basis, late blight, caused by the fungus *Phytophthora infestans*, is the most important of potato diseases. Worldwide losses due to potato late blight are estimated to be about \$3 billion annually. Conservatively, *P. infestans* costs the potato industry in the United States \$200 to \$400 million annually.

[0004] Currently, late blight is controlled by application of fungicides. The cost of chemical control in the U.S., now applied in essentially all potato producing regions, is approximately \$100-\$200 per acre. Given that approximately 1.2 million acres are planted to potatoes annually in the U.S., the control costs alone are significant. In addition, in many years storage losses due to this pathogen are in the same range as the cost of control.

[0005] In the U.S., the recent migration from Mexico of highly aggressive and virulent new forms of *P. infestans* poses a serious threat to all potato producing regions. In particular, the presence of A2 mating type and fungicide resistant forms in field populations of the fungus limits producers' options in control practices.

[0006] *P. infestans* also causes late blight in other crops, including tomato, eggplant, and other solanaceous species. The new, aggressive strains of *P. infestans* also represent a serious threat to commercial tomato production.

[0007] Identification of a late blight resistance gene and development of transgenic plants resistant to *P. infestans*, is important goal in plant research to reduce crop losses and to reduce the need for fungicide application and costs of chemical control.

[0008] A wide variety of genetic loci that confer resistance to pathogens have been identified in plant species. These resistance loci often encode dominant resistance genes, or R genes. The R genes confer either vertical race-specific or horizontal nonspecific resistance to a pathogen (Plank, 1968). Vertical resistance is based upon an induced hypersensitive response in which the pathogen infection is contained by localized host cell death at infection sites. The mechanism for vertical resistance has been proposed to involve activation of the cell death response when a specific plant receptor (the R gene product) interacts with an elicitor produced by a corresponding Avr gene in the invading pathogen (Flor, 1971).

Pathogen races are defined by distinct Avr gene profiles and resistance results from the interaction between specific R gene and Avr gene products (the gene for gene interaction).

[0009] In contrast to vertical resistance, horizontal resistance generally involves multiple plant genes and provides a general, stable, pathogen resistance in a race-nonspecific manner. Horizontal resistance is not correlated with the hypersensitive response, involving instead limiting pathogen spread in the host. *Solanum bulbocastanum* contains a dominant R gene locus which confers horizontal resistance to *P. infestans* when introgressed into the cultivated potato (Naess *et al.*, 2000; Naess *et al.*, 2001).

[0010] Map-based cloning has been employed to identify a variety of R genes from crop plants (Ballvora *et al.*, 2002; Brueggeman *et al.*, 2002; Dixon *et al.*, 1996; Feuillet *et al.*, 1997; Lagudah *et al.*, 1997; Ori *et al.*, 1997; Yoshimura *et al.*, 1998).

SUMMARY OF THE INVENTION

[0011] We have now isolated a gene from the wild potato species *Solanum bulbocastanum* which confers horizontal resistance to *Phytophthora infestans*, the fungal pathogen that causes late blight disease. cDNA and genomic DNA sequences of the *Solanum bulbocastanum* late blight resistance gene, hereinafter denoted as *Sbul1*, are specifically exemplified herein (SEQ ID NO:1 and 3, respectively). The deduced amino acid sequence is shown in SEQ ID NO:2 and 4. The resistance protein is in the class of Nucleotide Binding Site-Leucine-Rich Repeat Proteins (NBS-LRRP), and the gene in *S. bulbocastanum* is flanked by related NBS-LRRP gene sequences.

[0012] DNA encoding the resistance protein has been introduced into potato plants and confers resistance to *P. infestans*. A comparison of the deduced amino acid sequence of *Sbul1*, which confers late blight resistance in transgenic plants, and the deduced amino acid sequence encoded by the *S. bulbocastanum* gene denoted herein as *Sbul2*, which does not confer resistance, reveals 101 differences between the two proteins over 989 residues, or 90% identity. A comparison of the nucleic acid sequences of *Sbul1* and *Sbul2* reveals 221 differences between the two genes over 3174 bp of coding sequence, or 93% identity.

[0013] Accordingly, the invention is directed to nucleic acid molecules encoding a pathogen resistance gene, the gene being characterized in that it encodes the amino acid sequence shown in SEQ ID NO:4, or an amino acid sequence showing greater than about 90% sequence identity with SEQ ID NO:4 and which encodes a polypeptide having ability to confer or enhance a plant's resistance to late blight. Exemplary nucleic acid molecules include the exemplified cDNA and genomic DNA sequences and nucleic acid sequences

having greater than about 93% sequence identity with the coding domain of the exemplified sequences and which confer or enhance a plant's resistance to late blight.

[0014] The invention is also directed to recombinant nucleic acid molecules containing the sequences encoding the polypeptides which confer late blight resistance, including, for example, recombinant vectors, such as cloning, expression or transformation vectors.

[0015] Another aspect of the invention is the provision of cells which are transformed by the vectors or DNA sequences of the invention.

[0016] Methods of using the sequences are also encompassed by the invention. A particular use of the invention is the provision of plants or plant cells transformed with one or more nucleic acid sequences encoding a polypeptide which confers late blight resistance to provide plants having resistance to *P. infestans*, or to provide plants having enhanced resistance to *P. infestans* or related plant pathogens. Such plants include, for example, solanaceous plants. Prominent food crops are in the *Solanaceae* family. These include potato (*Solanum tuberosum*); tomato (*Lycopersicon*, e.g., *L. lycopersicum* and *L. esculentum*); pepper (*Capsicum*); eggplant (*Solanum melongena*). Most preferably, in the practice of the invention, the solanaceous plant is potato.

[0017] As described below, the locus containing the resistance gene was characterized by map-based cloning and chromosome walking using a *S. bulbocastanum* Bacterial Artificial Chromosome (BAC) library. The actual resistance gene was isolated using Polymerase Chain Reaction (PCR) as the allele of the locus which contains the gene was not represented in the library. Chimeric transgenes constructed with *Sbull* transcribed from a potato ubiquitin (*Ubi3*) promoter were introduced into a susceptible potato variety. Greenhouse tests confirmed that transgenic potato clones containing these transgenes are resistant to late blight.

[0018] Accordingly, it is an object of the invention to provide nucleic acid sequences encoding polypeptides that confer late blight resistance; isolated polypeptides having this

activity; recombinant nucleic acid molecules including expression vectors encoding the polypeptides; and cells harboring the recombinant nucleic acid molecules or expression vectors.

[0019] It is also an object of the invention to provide transformation vectors comprising a recombinant molecule, which vectors are effective for stably introducing the recombinant molecule into a plant.

[0020] It is also an object of the invention to provide methods of producing and using polypeptides conferring late blight resistance.

[0021] It is another object of the invention to provide transgenic plants having resistance to late blight or related pathogen, wherein the resistance is a result of expression of a recombinant nucleic acid molecule of the invention. An important aspect is the conferral of horizontal resistance to late blight, thereby providing general rather than race-specific control of the pathogen.

[0022] A further aspect of the invention is the provision of oligonucleotide probes capable of detecting a late blight resistance gene or functional equivalents thereof and the use of the probes to isolate nucleic acid sequences encoding a late blight resistance polypeptide or functional equivalent thereof.

[0023] A major impact of this invention on agriculture will be in controlling *P. infestans* in potatoes. The introduction of the resistance gene into cultivated potatoes would be expected to significantly reduce costs of chemical control, as well as providing a novel method for controlling fungicide resistant pathogen populations.

[0024] An additional application of this invention is controlling late blight in other solanaceous plants, for example, tomato production. The new, aggressive strains of *P. infestans* also represent a serious threat to commercial tomato production. Introduction of this resistance gene into tomato will result in significant savings in chemical control of the pathogen in this commodity.

[0025] Other objects and advantages of the invention will become readily apparent from the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1 shows the genetic map of the *S. bulbocastanum* late blight resistance gene locus. The approximate position of the locus is indicated by R. The positions of several RFLP markers relative to this locus are indicated. The relative positions of AFLP markers flanking the R gene are indicated.

[0027] FIG. 2 shows the assembly of an approximately 600 kb contig on *S. bulbocastanum* anchored by a BAC clone hybridizing to the RFLP marker CD60. BAC C29 was cloned by hybridization of filters to the labeled RFLP marker. BAC end-sequence analysis allowed design of specific primer pairs for both ends of the insert (F and R indicate forward and reverse). For each walk subsets of the BAC library were pooled and screened by PCR using these specific primers. BAC end-sequence analysis also revealed the position of members of a family of nucleotide binding site-leucine-rich repeat proteins (NBS-LRRP) indicated.

[0028] FIG. 3 shows the structure of the *S. bulbocastanum* chromosome 8 NBS-LRRP domain linked to late blight resistance. The domain contains six complete and three partial NBS-LRRP coding sequences. Only two of the six complete genes on the BAC contig, *Sbul2* and *Sbul3*, were found to encode uninterrupted open reading frames. The remaining four NBS-LRRP genes are interrupted by frame shift mutations (NBS Sal 37-1 and *Sbul1*) or stop codons (NBS Sal 37-3 and NBS 24K).

[0029] FIG. 4 shows the structure of the *Sbul1* transgenes. *Sbul1* genomic (SEQ ID NO:3) and cDNA (SEQ ID NO:1) sequences were fused to promoter and terminator sequences from the potato *Ubi3* gene (Garbarino *et al.*, 1994a; Garbarino *et al.*, 1994b).

[0030] FIG. 5 shows transgenic potatoes expressing *Sbul1* genomic and cDNA transgenes have improved resistance to *P. infestans* US8. Detached leaves of greenhouse-grown

transgenic and control plants were inoculated with *P. infestans* and incubated for four days. Lesion size determined computationally (Bioquant Systems).

[0031] FIG. 6 shows a comparison of the deduced amino acid sequences of *Sbul1*, which confers late blight resistance in transgenic plants, and *Sbul2* which does not. Comparison reveals 101 differences between the two proteins over 989 residues, or 90% identity.

[0032] FIG. 7 shows a comparison of the nucleic acid sequences of *Sbul1*, which confers late blight resistance in transgenic plants, and *Sbul2* which does not. Comparison reveals 221 differences between the two genes over 3174 bp of coding sequence, or 93% identity

[0033] FIG. 8 shows potato lines transformed with the *Sbul1* genomic transgene have enhanced resistance to *P. infestans* US8 in intact plant assays.

BRIEF DESCRIPTION OF THE SEQUENCES

[0034] SEQ ID NO:1 shows the cDNA sequence of the *Solanum bulbocastanum* late blight resistance gene *Sbul1*. Sequence feature information: *Solanum bulbocastanum Sbul1* cDNA sequence: nucleotide 1 to 3193; coding region: nucleotide 52 to 3018; translation initiation codon: nucleotide 52 to 54; translation termination codon: nucleotide 3016 to 3018.

[0035] SEQ ID NO:2 shows the amino acid sequence encoded by SEQ ID NO:1.

[0036] SEQ ID NO:3 shows the DNA sequence of the active *Sbul1* gene, a PCR product using template DNA from a late blight-resistant back cross 3 potato line containing *S. bulbocastanum* DNA. The sequence contains a 412 bp intron. Sequence feature information: *Solanum bulbocastanum* genomic *Sbul1* sequence: nucleotide 1 to 3595; coding region: first coding domain : nucleotide 57 to 487; second coding domain: nucleotide 900 to 3435, wherein the 5' end of the second domain is linked to the 3' end of the first domain; intron: nucleotide 488 to 899; translation initiation codon: nucleotide 57 to 59; translation termination codon: nucleotide 3433 to 3435.

[0037] SEQ ID NO:4 shows the amino acid sequence encoded by SEQ ID NO:3.

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[0038] SEQ ID NO:5 shows the DNA sequence of the *Sbul2* gene. Sequence feature information: *Solanum bulbocastanum* genomic *Sbul2* sequence: nucleotide 1 to 3347; coding region: first coding domain: nucleotide 57 to 509; second coding domain: nucleotide 789 to 3347, wherein the 5' end of the second domain is linked to the 3' end of the first domain; intron: nucleotide 510 to 788; translation initiation codon: nucleotide 57 to 59; translation termination codon: nucleotide 3345 to 3347.

[0039] SEQ ID NO:6 shows the amino acid sequence encoded by SEQ ID NO:5.

[0040] SEQ ID NO:7 shows the DNA sequence of the *Sbul3* gene. Sequence feature information: *Solanum bulbocastanum* genomic *Sbul3* sequence: nucleotide 1 to 3222; coding region: first coding domain : nucleotide 58 to 528; second coding domain: nucleotide 691 to 3222, wherein the 5' end of the second domain is linked to the 3' end of the first domain; intron: nucleotide 529 to 690; translation initiation codon: nucleotide 58 to 60; translation termination codon: nucleotide 3220 to 3222.

[0041] SEQ ID NO:8 shows the amino acid sequence encoded by SEQ ID NO:7.

[0042] SEQ ID NO:9 shows the sequence of the chimeric *Ubi3/Sbull* genomic transgene. Sequence feature information: *Ubi3-Solanum bulbocastanum* genomic *Sbull-Ubi3* sequence: nucleotide 1 to 5028; Potato *Ubi3* promoter: nucleotide 1 to 953; *Solanum bulbocastanum* genomic *Sbull* gene: nucleotide 973 to 4566; coding region: first coding domain : nucleotide 1029 to 1459; second coding domain: nucleotide 1872 to 4407, wherein the 5' end of the second domain is linked to the 3' end of the first domain; intron: nucleotide 1460 to 1871; translation initiation codon: nucleotide 1029 to 1031; translation termination codon: nucleotide 4405 to 4407.

[0043] SEQ ID NO:10 shows the amino acid sequence encoded by SEQ ID NO:9.

DEFINITIONS

[0044] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs.

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The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton, *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY (2d ed. 1994); THE CAMBRIDGE DICTIONARY OF SCIENCE AND TECHNOLOGY (Walker ed., 1988); THE GLOSSARY OF GENETICS, 5TH ED., Rieger, R., *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY (1991). References providing standard molecular biological procedures include Sambrook *et al.* (1989) *Molecular Cloning*, second edition, Cold Spring Harbor Laboratory, Plainview, NY; *DNA Cloning*, Vols. I and II, IRL Press, Oxford, UK; and Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK. References related to the manipulation and transformation of plant tissue include Kung and Arntzen (eds.) (1989) *Plant Biotechnology*, Butterworths, Stoneham, MA; R. A. Dixon (ed.) (1985) *Plant Cell Culture: A Practical Approach*, IRL Press, Oxford, UK; Schuler and Zielinski (1989) *Methods in Plant Molecular Biology*, Academic Press, San Diego, CA; Weissbach and Weissbach (eds.) (1988) Academic Press, San Diego, CA; I. Potrykus (1991) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:205; Weising *et al.* (1988) *Annu. Rev. Genet.* 22:421; van Wordragen *et al.* (1992) *Plant Mol. Biol. Rep.* 19:12; Davey *et al.* (1989) *Plant Mol. Biol.* 13:273; Walden and Schell (1990) *Eur. J. Biochem.* 192:563; Joersbo and Brunstedt (1991) *Physiol. Plant.* 81:256 and references cited in those references. The references cited in the list of References attached below also provides a description of the terms used herein. The following U.S. patents are incorporated herein by reference: U.S. Patents Nos. 5,589,339; 6,084,156; 6,225,527; 6,287,865; 6,225,532; 6,287,865; 6,100,449; and published application PCT/US00/23802 (WO 01/16353). All references cited in the present application are expressly incorporated by reference herein.

DETAILED DESCRIPTION OF THE INVENTION

[0045] We have now cloned a horizontal late blight resistance gene from *S. bulbocastanum*. As described below, the resistance gene *Sbull* was isolated by map-based cloning. In this technique the locus that confers resistance is mapped relative to amplified fragment length

polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers that are linked to the resistance gene. Four markers that appeared to be most closely linked to the resistance gene were used to probe a *S. bulbocastanum* genomic bacterial artificial chromosome (BAC) library and hybridizing BAC clones identified. The resistance locus was obtained by chromosome walking from an original anchor clone. The resistance gene was identified by introduction of candidate genes from the locus into transgenic potato and screening for late blight resistance.

[0046] The present invention is directed to isolated nucleic acid sequences derived from a *S. bulbocastanum* gene which encode polypeptides which confer horizontal late blight resistance. The specifically exemplified nucleic acid sequences include the *Sbul1* cDNA sequence (SEQ ID NO:1) and the DNA sequence of the active *Sbul1* gene, a PCR product using template DNA from a late blight-resistant back cross 3 potato line containing *S. bulbocastanum* DNA (SEQ ID NO:3). The latter sequence contains a 412 bp intron. SEQ ID NO:4 shows the deduced amino acid sequence of the *Sbul1* gene product. The invention encompasses nucleic acid sequences which have greater than about 93% sequence identity with the coding domain of the exemplified sequences and encode a polypeptide which confers or enhances a plant's resistance to late blight. More preferably, the nucleic acid sequences have about 95% sequence identity with the coding domain of the exemplified sequences and encode a polypeptide which confers or enhances a plant's resistance to late blight. For purposes of the present invention, the degree of identity between two nucleic acid sequences is determined any method known in the art, for example by the Clustal method (Thompson *et al.* 1994), using ClustalW 1.7 or 1.8 (<http://dot.imgen.bcm.tmc.edu:9331/multi-align/multi-align.html>). Further, nucleic acid sequences which hybridize under high stringency conditions with the coding region of the DNA sequence of SEQ ID NO:1 or 3 and which encode a polypeptide having the activity defined above, are also encompassed by the present invention.

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[0047] The invention is directed to nucleic acid molecules encoding the amino acid sequence of SEQ ID NO:4, or an amino acid sequence showing greater than about 90% sequence identity with SEQ ID NO:4 and which encodes a polypeptide having ability to confer or enhance a plant's resistance to late blight. More preferably, the encoded amino acid sequence has at least about 95%, and most preferably at least about 97% sequence identity with SEQ ID NO:4 and has the activity defined above. For purposes of the present invention, the degree of identity between two amino acids is determined any method known in the art, for example, by the FASTA/FASTP method of Pearson (1990), using ALIGN (<http://dot.imgen.bcm.tmc.edu:9331/seq-search/alignment.html>), with the BLOSUM50 or PAM250 scoring matrix.

[0048] Preferably, the polypeptides of the present invention comprise an amino acid sequence of SEQ ID NO:4 or an amino acid sequence showing greater than about 90% sequence identity with SEQ ID NO:4 and which encodes a polypeptide having ability to confer or enhance a plant's resistance to late blight.

[0049] The degeneracy of the genetic code is well known to the art; therefore, synonymous coding sequences with one or more codon substitutions can be readily determined by one of ordinary skill in the art. Synonymous coding sequences vary from the exemplified coding sequences but encode proteins of the same amino acid sequences as those specifically provided herein. Examples of conservative substitutions are within the groups of basic amino acids (such as arginine, lysine and histidine), acidic amino acids (such as glutamic acid and aspartic acid), polar amino acids (such as glutamine and asparagine), hydrophobic amino acids (such as leucine isoleucine and valine), aromatic amino acids (such as phenylalanine, tryptophan and tyrosine), and small amino acids (such as glycine, alanine, serine, threonine and methionine). Amino acid substitutions which do not generally alter the specific activity are known in the art as described, for example, by H. Neurath and R. L. Hill, 1979, *In, The Proteins*, Academic Press, New York. The most commonly occurring exchanges are Ala/Ser,

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Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly as well as these in reverse.

[0050] The present invention also relates to recombinant expression vectors comprising a nucleic acid sequence of the present invention, a promoter, and transcriptional and translational stop signals.

[0051] The present invention also relates to recombinant host cells, comprising a nucleic acid sequence of the invention, which are advantageously used in the recombinant production of the polypeptides. Preparation of transformed host cells and cloning methods are described by U.S. Patent No. 5,374,540, which is incorporated herein by reference.

[0052] Preparation of Transgenic Plants: The transgenic plant or plant cell expressing an RNA transcript or polypeptide of the present invention may be constructed in accordance with methods known in the art. In brief, the plant or plant cell is constructed by incorporating one or more expression constructs encoding a polypeptide of the present invention into the plant host genome and propagating the resulting modified plant or plant cell into a transgenic plant or plant cell.

[0053] As discussed above, a particular use of the invention is the provision of plants or plant cells transformed with a DNA sequence encoding an amino acid sequence which confers resistance to late blight or related pathogens.

[0054] Another use of the invention is as probes and primers capable of detecting a late blight resistance gene or functional equivalent thereof in fungi of the genus *Phytophthora*. Using the nucleic acid sequences of the invention facilitates the isolation of homologous genes from hosts to obtain genes which protect host cells, including fungi and plants against other fungal pathogens.

EXAMPLES

[0055] The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention.

Map-based cloning of the *S. bulbocastanum* late blight resistance gene (*Sbull*)

[0056] *S. bulbocastanum* DNA was introgressed into potato by somatic fusion at the University of Wisconsin (Naess *et al.*, 2001). Fertile progeny were then back crossed to potato. The position of the *S. bulbocastanum* late blight resistance gene locus was mapped using a back-cross 3 population segregating for *P. infestans* resistance using a combination of AFLP (Vos *et al.*, 1995) and RFLP techniques. The late blight resistance locus maps to chromosome 8 (Naess *et al.*, 2001). The segregating population was subjected to AFLP mapping, exhaustion of the commercially available primer/enzyme sets resulted in identification of over 400 polymorphic bands. RFLP mapping was also employed, the population was screened with a variety of chromosome 8 markers. The relative positions of the AFLP and RFLP markers closest to the *Sbull* locus are shown in FIG. 1. The clustering of these markers, together with the failure of AFLP to generate a marker within the flanking RFLP probes (CD60 and TG261) suggested that the resistance locus is located in an area of chromosome 8 with high rates of recombination resulting in very different genetic and physical maps. This interpretation suggested that additional mapping was unnecessary, and four RFLP markers (TG282, TG505, CD60, PPOIII) were selected to probe a *S. bulbocastanum* BAC library (Song *et al.*, 2000).

Identification of Candidate *Sbull* genes.

[0057] BAC clones corresponding to each of the four RFLP markers were isolated and used to anchor PCR-based chromosome walking (FIG. 1). BAC end-sequences were used to generate specific primer pairs for screening of pooled BAC clones by PCR (Cai *et al.*, 1995). The assembly of an approximately 600 kb contig proximal to the CD60 RFLP marker on *S. bulbocastanum* chromosome 8 is shown in FIG. 2. Computational (BLAST) alignment of the end sequences of BAC isolates C29F2F2R1 and C29F2F2R2 with the available database (Altschul *et al.*, 1990) indicated the presence of sequences encoding nucleotide binding site-leucine-rich repeat proteins (NBS-LRRPs) similar to previously identified R genes (Ballvora *et al.*, 2002; Lagudah *et al.*, 1997; Simons *et al.*, 1998; Yoshimura *et al.*, 1998). Primers

specific to the NBS-LRRP locus on the contig in FIG. 2 were employed in PCR screening of genomic DNA from the original population segregating for late blight resistance, and this locus was found to be linked to the resistance phenotype.

[0058] An approximately 75 kb region containing six complete NBS-LRRP genes was characterized. As shown in FIG. 3, four of the six complete genes were found to represent pseudogenes, with coding sequences interrupted by either frame shift mutations or stop codons. These data suggested that late blight resistance at this locus was associated with *Sbul2* and/or *Sbul3* expression.

Identification of the *Sbul1* late blight resistance gene.

[0059] Experiments to determine the efficacy of either *Sbul2* or *Sbul3* (FIG. 3) in conferring late blight resistance were based on mobilization of these genes plus at least 3 kb of 5' and 3' flanking sequence into susceptible potatoes by *Agrobacterium*-mediated transformation. *Sbul2* or *Sbul3* and flanking sequences were mobilized into a binary transformation vector pCGN1547 (McBride *et al.*, 1990). These binary vector constructs were used to introduce the *Sbul2* or *Sbul3* genes into potato varieties Lenape (Akeley *et al.*, 1968) and Atlantic (Webb *et al.*, 1978) by a standard transformation/selection protocol (Snyder *et al.*, 1993). Transgenic potato plants containing either the *Sbul2* or *Sbul3* genes were screened for resistance to late blight by detached leaf assay (Trognitz *et al.*, 1995). Neither the *Sbul2* or *Sbul3* genes conferred resistance to *P. infestans*.

[0060] The similarity of the NBS-LRRPs on the *S. bulbocastanum* contig (FIG. 3) to known disease resistance genes is significant. A BLAST database search (Altschul *et al.*, 1990) using the deduced amino acid sequence of *Sbul2* returns seven putative resistance genes from *Arabidopsis* at the highest identity ($P(N) < 10^{-120}$) followed by the *I2 Fusarium oxysporum* resistance gene from tomato (Simons *et al.*, 1998) ($P(N) < 10^{-108}$). In addition, this PCR probes from this locus indicate linkage to the resistance gene in the segregating population. It therefore appeared possible that one or more of the four pseudogenes present

on the *S. bulbocastanum* contig (FIG. 3) represented an inactive allele of a gene active on the other chromosome of this diploid species. Specific primers were prepared to the *Sbul1*, *Sbul2*, and *Sbul3* genes on the locus, and RACE (Rapid Amplification of cDNA Ends)-PCR was employed to amplify potential mRNAs from polyA⁺ RNA prepared from *P. infestans*-infected *S. bulbocastanum* leaves. Messenger RNA products corresponding to *Sbul1*, *Sbul2* and *Sbul3* were amplified. This suggested that active *Sbul1* was heterozygous in *S. bulbocastanum*, with one allele active and the other interrupted by a frame shift mutation (Helgeson *et al.*, 1988). PCR amplification of *Sbul1* using genomic DNA from a late blight-resistant BC3 line as a template generated an amplified product encoding a mRNA essentially identical to the *Sbul1* cDNA (SEQ ID NO:3).

[0061] The DNA sequence of the active *Sbul1* cDNA is shown in SEQ ID NO:1. The deduced amino acid sequence is shown in SEQ ID NO:2. The DNA sequence of active *Sbul1* gene, a PCR product from *S. bulbocastanum*-containing potato genomic DNA, containing a 412 bp intron is shown in SEQ ID NO:3. The deduced amino acid sequence of the *Sbul1* gene product is shown in SEQ ID NO:4. The DNA sequence of the *Sbul2* gene is shown in SEQ ID NO:5, and the deduced *Sbul2* amino acid sequence is shown in SEQ ID NO:6. The DNA sequence of the *Sbul3* gene is shown in SEQ ID NO:7, and the deduced *Sbul3* amino acid sequence is shown in SEQ ID NO:8.

Expression of *Sbul1* in transgenic plants

[0062] In order to express *Sbul1* in transgenic plants two chimeric transgenes were constructed. Transcription of the *Sbul1* gene is directed from the potato *Ubi3* promoter, which will result in constitutive moderate-level expression (Garbarino *et al.*, 1994a; Garbarino *et al.*, 1994b). The *Ubi3* polyadenylation signal was fused to the 3' end of each sequence (FIG. 4).

[0063] The sequence of the genomic chimeric transgene is shown in SEQ ID NO:9. The transgenes shown in FIG. 4 were mobilized into the binary transformation vector pBINPLUS-

ARS. This vector is a version of the pBINPLUS vector (Van Engelen *et al.*, 1995) modified in our laboratory by replacement of selectable marker transcriptional control sequences (CaMV35S promoter, NOS terminator) with a promoter and terminator derived from the potato *Ubi3* gene (Garbarino *et al.*, 1994a). These binary vector constructs were used to introduce the transgenes into potato varieties Lenape (Akeley *et al.*, 1968) and Atlantic (Webb *et al.*, 1978) by a standard transformation/selection protocol (Snyder *et al.*, 1993). Transgenic potato plants were screened for resistance to late blight by detached leaf assay (Trognitz *et al.*, 1995).

Greenhouse assay of late blight resistance of transgenic potatoes expressing *Sbul1* transgenes

[0064] To assay for late blight resistance fully developed leaves from greenhouse-grown plants were detached. Inocula were obtained from two-week-old cultures of *P. infestans* (strain US8, Florida isolate) grown on rye agar. Inoculations were made by placing a 10ul droplet of a sporangial suspension (4×10^4 ml) that had been incubated at 8° C for 2.5 hours (to liberate zoospores) on both sides of the midrib of the abaxial surface. The inoculated leaflets were placed in petri dishes containing moistened filter paper to maintain 100% relative humidity. Inoculated material was incubated for 1 day at 15°C in darkness, then for four days at 15°C, 16-hour/day photoperiod ($400 \text{ E} \cdot \text{m}^{-2} \cdot \text{S}^{-1}$). A computer-driven image analysis system (Bioquant IV, R and M Biometrics, Nashville, TN) was used to obtain measurements of lesions. The lesion diameter was determined by projecting the whole leaves onto a grid lining the Bioquant Digitizing Pad. The digitizing pad was coupled with an IBM PC and measurements were generated using Bioquant Systems software.

[0065] As shown in FIG. 5, both the *Sbul1* genomic and cDNA transgenes conferred resistance to *P. infestans* in transgenic potatoes. As shown in FIG. 6, the deduced amino acid sequence of the *Sbul2* gene, which does not confer resistance, has 90% identity to the *Sbul1* deduced amino acid sequence. As shown in FIG. 7, the nucleic acid sequences of the *Sbul1* and *Sbul2* coding domains are 93% identical.

[0066] The data presented in FIG. 5 shows that *Sbul1*, when introduced into susceptible potato varieties, is capable of conferring resistance to late blight. While the *Sbul2* and *Sbul3* genes do not, individually, confer a resistant phenotype, this does not preclude a role for these gene products in enhancing *Sbul1*-mediated resistance originating from this locus.

Whole-plant glasshouse test of late blight resistance of potato plants transformed with *Sbul1*.

[0067] To assay for late blight resistance, transgenic and control tubers were planted in 6 inch pots and grown 16 hr light and 8 hr dark photoperiod using high pressure sodium lamps as supplemental lighting. Transgenic lines used in these experiments contained the genomic *Sbul1* transgene (SEQ ID NO:9). Inocula were obtained from cultures of *P. infestans* (strain MD-02-pet-1 an A2, US-8 genotype) grown in lima bean media in the dark at room temperature. After two weeks of incubation, the plates were flooded 2x with sterile water and scraped lightly using an L-shaped glass or plastic rod to collect sporangia. The liquid from the plates were filtered into a 1 liter glass beaker using two layers of cheesecloth. The total volume was roughly estimated and sporangia was counted using a hemacytometer. Using sterile water, the volume of the inoculum was adjusted that gave a final count of 5,000 sporangia/ml. The inoculum was transferred into a sprayer (approximately 2 ml/sec) and incubated at 4°C for 1 hour followed by room temperature incubation for 30 minutes.

[0068] The whole-plant glasshouse test described by Stewart et al. (1983) was used to determine which of the plants were resistant to *P. infestans*. Plants of each clone in flower bud were inoculated with *P. infestans*. Each plant was scored daily using Malcolmson's scoring scale of increasing resistance (Cruickshank et al., 1982) starting 7 days after inoculation, and plants of each clone compared. As shown in FIG. 8, two of the transgenic lines exhibited no infection 24 days after inoculation, six additional transgenic lines had intermediate levels of resistance.

Description of Plasmids

[0069] The plasmid pBT1596 consists of the *Sbull* genomic transgene shown in SEQ ID NO:9 inserted into the multiple cloning site of the binary transformation vector pBINPLUS-ARS. The plasmid pBT1593 consists of the *Sbull* cDNA sequence (SEQ ID NO:1) inserted between the potato *Ubi3* promoter and terminator sequences indicated in SEQ ID NO:9 in the multiple cloning site of the binary transformation vector pBINPLUS-ARS.

Statement of Deposit

[0070] The plasmids were introduced into the host *Escherichia coli* DH5a and the transformed *Escherichia coli* strains were deposited August 18, 2003 under terms of the Budapest Treaty with Agricultural Research Service Culture Collection (NRRL) National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604 USA and given the following accession numbers:

<u>Plasmid</u>	<u>Accession No.</u>	<u>SEQ ID NO</u>
pBT1596	NRRL B-30685	SEQ ID NO:9
pBT1593	NRRL B-30686	SEQ ID NO:1

[0071] It is understood that the foregoing detailed description is given merely by way of illustration and that modification and variations may be made within, without departing from the spirit and scope of the invention. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety.

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SEQUENCE LISTING

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Lys Glu Ile Val Glu Ser Ile Glu Glu Lys Ser Leu Gly Gly Met Asp		
	225	230 235 240
Leu Ala Pro Leu Gln Lys Lys Leu Arg Asp Leu Leu Asn Gly Lys Lys		
	245	250 255
Tyr Leu Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Asp Lys Trp		
	260	265 270
Ala Lys Leu Arg Gln Val Leu Lys Val Gly Ala Ser Gly Ala Ser Val		
	275	280 285
Leu Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu		
	290	295 300

Gln Pro Tyr Glu Leu Ser Asn Leu Ser Gln Glu Asp Cys Trp Leu Leu
 305 310 315 320

Phe Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn Leu Asn Leu
 325 330 335

Val Ala Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu
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Ala Ala Lys Thr Leu Gly Gly Ile Leu Arg Phe Lys Arg Glu Glu Arg
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Gln Trp Glu His Val Arg Asp Ser Glu Ile Trp Lys Leu Pro Gln Glu
 370 375 380

Glu Ser Ser Ile Leu Pro Ala Leu Arg Leu Ser Tyr His His Leu Pro
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Leu Asp Leu Arg Gln Cys Phe Thr Tyr Cys Ala Val Phe Pro Lys Asp
 405 410 415

Thr Glu Met Glu Lys Gly Asn Leu Ile Ser Leu Trp Met Ala His Gly
 420 425 430

Phe Ile Leu Ser Lys Gly Asn Leu Glu Leu Glu Asn Val Gly Asn Glu
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Val Trp Asn Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val
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Lys Ser Gly Gln Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu
 465 470 475 480

Ala Thr Ser Leu Phe Ser Ala Ser Thr Ser Ser Ser Asn Ile Arg Glu
 485 490 495

Ile Ile Val Glu Asn Tyr Ile His Met Met Ser Ile Gly Phe Thr Lys
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Val Val Ser Ser Tyr Ser Leu Ser His Leu Gln Lys Phe Val Ser Leu
 515 520 525

Arg Val Leu Asn Leu Ser Asp Ile Lys Leu Lys Gln Leu Pro Ser Ser
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Ile Gly Asp Leu Val His Leu Arg Tyr Leu Asn Leu Ser Gly Asn Thr
 545 550 555 560

Ser Ile Arg Ser Leu Pro Asn Gln Leu Cys Lys Leu Gln Asn Leu Gln
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Thr Leu Asp Leu His Gly Cys His Ser Leu Cys Cys Leu Pro Lys Glu
 580 585 590

Thr Ser Lys Leu Gly Ser Leu Arg Asn Leu Leu Leu Asp Gly Cys Tyr
 595 600 605

Gly Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys
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Thr Leu Ser Arg Phe Val Val Gly Ile Gln Lys Lys Ser Cys Gln Leu
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Gly Glu Leu Arg Asn Leu Asn Leu Tyr Gly Ser Ile Glu Ile Thr His
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Leu Glu Arg Val Lys Asn Asp Met Asp Ala Lys Glu Ala Asn Leu Ser
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Ala Lys Glu Asn Leu His Ser Leu Ser Met Lys Trp Asp Asp Asp Glu
 675 680 685

Arg Pro Arg Ile Tyr Glu Ser Glu Lys Val Glu Val Leu Glu Ala Leu
 690 695 700

Lys Pro His Ser Asn Leu Thr Cys Leu Thr Ile Arg Gly Phe Arg Gly
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Ile Arg Leu Pro Asp Trp Met Asn His Ser Val Leu Lys Asn Val Val
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Ser Ile Glu Ile Ile Ser Cys Lys Asn Cys Ser Cys Leu Pro Pro Phe
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Glu Val Glu Tyr Val Asp Ser Gly Phe Pro Thr Arg Arg Arg Phe Pro
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Ser Leu Arg Lys Leu Asn Ile Arg Glu Phe Gly Asn Leu Lys Gly Leu
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 Lys Leu Val Val Ser Gly Asp Lys Ser Asp Ala Ile Gly Phe Ser Ser
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 Lys Tyr Leu Asn Ile Ser Phe Tyr Phe Asn Leu Lys Glu Leu Pro Thr
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 Ser Leu Ala Ser Leu Asn Ala Leu Lys His Leu Glu Ile His Ser Cys
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Gln Gly Glu Leu Gly Leu Ile Leu Gly Phe Lys Asp Glu Phe Glu Lys
                20                      25                      30

ctt caa agc acg ttt act aca atc caa gct gtg cta gaa gat gct cag      203
Leu Gln Ser Thr Phe Thr Thr Ile Gln Ala Val Leu Glu Asp Ala Gln
                35                      40                      45

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Asn Ala Ala Ala Tyr Glu Ala Asp Asp Ile Leu Asp Glu Cys Lys Thr
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gag gca cca att aga cag aag aag aac aaa tat ggg tgt tat cat cca      347
Glu Ala Pro Ile Arg Gln Lys Lys Asn Lys Tyr Gly Cys Tyr His Pro
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aac gtt atc act ttt cgt cac aag att ggg aaa agg atg aaa aag att      395
Asn Val Ile Thr Phe Arg His Lys Ile Gly Lys Arg Met Lys Lys Ile
                100                      105                      110

atg gag aaa cta gat gta att gca gcg gaa cga att aag ttt cat ttg      443
Met Glu Lys Leu Asp Val Ile Ala Ala Glu Arg Ile Lys Phe His Leu
                115                      120                      125

gat gaa agg act ata gag aga caa gtt gct aca cgc caa aca gg      487
Asp Glu Arg Thr Ile Glu Arg Gln Val Ala Thr Arg Gln Thr Gly
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tgctcatctt agatattttt ctgaaaaaac agctttatat catcaaattc atgtgtgttt      547

tggaattcg tctaattctaa atgttcgtct caagtctaag tagataagtg gatccagctt      607

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gataaatcca tagcttactc ataggattag gataggcccc caagtctaaa tgacaggata      727

aagccagagt tgttttagct cttataaatt aacaatgata ataatgtgaa ttcaaaaaag      787
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gtgcaaaaatt ctactttgta tttttgctga ctctaccga gcttgggccca gg t ttt	903
	Phe 145
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Val Leu Asn Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp Glu	
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ata gtg aaa atc ctg ata aac aat gtt agc aat gcc caa aca ctt cca	999
Ile Val Lys Ile Leu Ile Asn Asn Val Ser Asn Ala Gln Thr Leu Pro	
	165 170 175
gtc ctc cca ata ctt ggt atg ggg gga cta gga aag acg act ctt gcc	1047
Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ala	
	180 185 190
caa atg gtc ttc aat gat cag aga gta att gag cat ttc cat ccc aaa	1095
Gln Met Val Phe Asn Asp Gln Arg Val Ile Glu His Phe His Pro Lys	
	195 200 205
ata tgg att tgt gtc tcg gaa gat ttt aat gag aag agg ttg ata aag	1143
Ile Trp Ile Cys Val Ser Glu Asp Phe Asn Glu Lys Arg Leu Ile Lys	
	210 215 220 225
gaa att gta gaa tct att gaa gaa aag tca ctt ggt ggc atg gac ttg	1191
Glu Ile Val Glu Ser Ile Glu Glu Lys Ser Leu Gly Gly Met Asp Leu	
	230 235 240
gct cca ctt caa aag aag ctt cgg gac ttg ctg aat gga aaa aaa tat	1239
Ala Pro Leu Gln Lys Lys Leu Arg Asp Leu Leu Asn Gly Lys Lys Tyr	
	245 250 255
ttg ctc gtc tta gat gat gtt tgg aat gaa gat caa gat aag tgg gct	1287
Leu Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Asp Lys Trp Ala	
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Lys Leu Arg Gln Val Leu Lys Val Gly Ala Ser Gly Ala Ser Val Leu	
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Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu Gln	
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cca tat gaa ttg tca aat ttg tct caa gaa gat tgt tgg ttg ttg ttc	1431
Pro Tyr Glu Leu Ser Asn Leu Ser Gln Glu Asp Cys Trp Leu Leu Phe	
	310 315 320
atg caa cgt gca ttt ggg cac caa gaa gaa ata aat ctt aat ctt gtg	1479
Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn Leu Asn Leu Val	
	325 330 335
gct atc gga aag gag att gtg aaa aaa tgt ggt ggt gtg cct cta gca	1527
Ala Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu Ala	
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gct aaa act ctt gga ggt att ttg cgc ttt aag aga gaa gaa aga cag	1575
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gat ttg aga caa tgc ttt aca tat tgt gca gta ttc cca aag gat acc Asp Leu Arg Gln Cys Phe Thr Tyr Cys Ala Val Phe Pro Lys Asp Thr 405 410 415	1719
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ctg aga aaa ctt aat ata cgc gaa ttt gat aat ctg aaa gga ttg ctg Leu Arg Lys Leu Asn Ile Arg Glu Phe Asp Asn Leu Lys Gly Leu Leu 790 795 800	2871
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tct aat ctc atg gct ctt act tcc ctc caa att cgc tat aac aaa gaa	3063

Ser Asn Leu Met Ala Leu Thr Ser Leu Gln Ile Arg Tyr Asn Lys Glu
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 ctg gct agt ctc aat gct ttg aag cat ctg gaa att cat agt tgt tat 3207
 Leu Ala Ser Leu Asn Ala Leu Lys His Leu Glu Ile His Ser Cys Tyr
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 Thr Gln Leu Ser Ile Thr Tyr Cys Glu Met Leu Gln Cys Leu Pro Glu
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 Pro Thr Leu Ala Lys Arg Cys Glu Lys Gly Ile Gly Glu Asp Trp Tyr
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Lys Leu Gln Ser Thr Phe Thr Thr Ile Gln Ala Val Leu Glu Asp Ala
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Gln Lys Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys
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Leu Asn Ala Ala Ala Tyr Glu Ala Asp Asp Ile Leu Asp Glu Cys Lys
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Thr Glu Ala Pro Ile Arg Gln Lys Lys Asn Lys Tyr Gly Cys Tyr His
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Pro Asn Val Ile Thr Phe Arg His Lys Ile Gly Lys Arg Met Lys Lys
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Ile Met Glu Lys Leu Asp Val Ile Ala Ala Glu Arg Ile Lys Phe His
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Leu Asp Glu Arg Thr Ile Glu Arg Gln Val Ala Thr Arg Gln Thr Gly
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Phe Val Leu Asn Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp
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Glu Ile Val Lys Ile Leu Ile Asn Asn Val Ser Asn Ala Gln Thr Leu
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Pro Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu
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Ala Gln Met Val Phe Asn Asp Gln Arg Val Ile Glu His Phe His Pro
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Lys Ile Trp Ile Cys Val Ser Glu Asp Phe Asn Glu Lys Arg Leu Ile
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Lys Glu Ile Val Glu Ser Ile Glu Glu Lys Ser Leu Gly Gly Met Asp
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Leu Ala Pro Leu Gln Lys Lys Leu Arg Asp Leu Leu Asn Gly Lys Lys
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Tyr Leu Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Asp Lys Trp
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Ala Lys Leu Arg Gln Val Leu Lys Val Gly Ala Ser Gly Ala Ser Val
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Leu Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu

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Phe Met Gln Arg Ala Phe 325	Gly His Gln Glu Glu 330	Ile Asn Leu Asn Leu 335
Val Ala Ile Gly Lys Glu 340	Ile Val Lys Lys Cys Gly 345	Gly Val Pro Leu 350
Ala Ala Lys Thr Leu Gly 355	Gly Ile Leu Arg Phe Lys 360	Arg Glu Glu Arg 365
Gln Trp Glu His Val Arg 370	Asp Ser Glu Ile Trp Lys 375 380	Leu Pro Gln Glu
Glu Ser Ser Ile Leu Pro 385	Ala Leu Arg Leu Ser Tyr 390 395	His His Leu Pro 400
Leu Asp Leu Arg Gln Cys 405	Phe Thr Tyr Cys Ala Val 410	Phe Pro Lys Asp 415
Thr Glu Met Glu Lys Gly 420	Asn Leu Ile Ser Leu Trp 425	Met Ala His Gly 430
Phe Ile Leu Ser Lys Gly 435	Asn Leu Glu Leu Glu Asn 440	Val Gly Asn Glu 445
Val Trp Asn Glu Leu Tyr 450	Leu Arg Ser Phe Phe Gln 455 460	Glu Ile Glu Val
Lys Ser Gly Gln Thr Tyr 465	Phe Lys Met His Asp Leu 470 475	Ile His Asp Leu 480
Ala Thr Ser Leu Phe Ser 485	Ala Ser Thr Ser Ser Ser 490	Asn Ile Arg Glu 495
Ile Ile Val Glu Asn Tyr 500	Ile His Met Met Ser Ile 505	Gly Phe Thr Lys 510
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Ile Gly Asp Leu Val His Leu Arg Tyr Leu Asn Leu Ser Gly Asn Thr
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 Ser Ile Arg Ser Leu Pro Asn Gln Leu Cys Lys Leu Gln Asn Leu Gln
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 580 585 590
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 595 600 605
 Gly Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys
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 Ala Lys Glu Asn Leu His Ser Leu Ser Met Lys Trp Asp Asp Asp Glu
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 Arg Pro Arg Ile Tyr Glu Ser Glu Lys Val Glu Val Leu Glu Ala Leu
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 705 710 715 720
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Ser Leu Arg Lys Leu Asn Ile Arg Glu Phe Asp Asn Leu Lys Gly Leu
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Ile Lys Cys Cys Pro Met Phe Val Ile Pro Thr Leu Ser Ser Val Lys
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Lys Leu Val Val Ser Gly Asp Lys Ser Asp Ala Ile Gly Phe Ser Ser
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Ile Ser Asn Leu Met Ala Leu Thr Ser Leu Gln Ile Arg Tyr Asn Lys
 850 855 860

Glu Asp Ala Ser Leu Pro Glu Glu Met Phe Lys Ser Leu Ala Asn Leu
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Lys Tyr Leu Asn Ile Ser Phe Tyr Phe Asn Leu Lys Glu Leu Pro Thr
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Ser Leu Ala Ser Leu Asn Ala Leu Lys His Leu Glu Ile His Ser Cys
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Tyr Ala Leu Glu Ser Leu Pro Glu Glu Gly Val Lys Gly Leu Ile Ser
 915 920 925

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Glu Gly Leu Gln His Leu Thr Ala Leu Thr Asn Leu Ser Val Glu Phe
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caa ggg gaa gtt gga ttg att ctt ggt ttt aag gat gag ttc gaa aag      155
Gln Gly Glu Val Gly Leu Ile Leu Gly Phe Lys Asp Glu Phe Glu Lys
      20                                25                                30

ctt caa agc aca ttt act aca atc caa gct gtg cta gaa gat gct cag      203
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aag aag caa ttg aag gac aag gca ata gaa aat tgg ttg cag aaa ctc      251
Lys Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys Leu
      50                                55                                60                                65

aat gct gct gta tat gaa gct gac gac atc ttg gac gaa tgt aaa act      299
Asn Ala Ala Val Tyr Glu Ala Asp Asp Ile Leu Asp Glu Cys Lys Thr
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gag gca cca att aga cag aag aag aac aaa tat ggg tgt tat cat cca      347
Glu Ala Pro Ile Arg Gln Lys Lys Asn Lys Tyr Gly Cys Tyr His Pro
      85                                90                                95

aac gtt atc gct ttc cgt cac aag att ggg aaa agg atg aaa aag att      395
Asn Val Ile Ala Phe Arg His Lys Ile Gly Lys Arg Met Lys Lys Ile
      100                                105                                110

atg gag aaa cta gat gta att gca gcg gaa cga att aag ttt cat ttg      443
Met Glu Lys Leu Asp Val Ile Ala Ala Glu Arg Ile Lys Phe His Leu
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Ala Glu Arg Thr Thr Glu Arg Gln Val Ala Thr Arg Gln Thr Gly Ala
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His Leu Arg Tyr Phe Ser
      150

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cagatttgga tatattaata tattatctaa atttgtttcg tgaaattttt aacagataaa      659

gcctgagttg ttttagacat tataaattaa caatgataat aatgtgaatt caaaaaagtg      719
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Leu Thr Pro Thr Glu Leu Gly Pro Gly Phe Val Leu Asn Glu	
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Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ala Gln Met Val Phe	
200 205 210	
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Asn Asp Gln Arg Val Ile Glu His Phe Leu Pro Lys Ile Trp Ile Cys	
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Val Ser Glu Asp Phe Asn Glu Lys Arg Leu Ile Lys Glu Ile Val Glu	
230 235 240 245	
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Ser Ile Glu Glu Lys Ser Leu Gly Asp Met Asp Leu Ala Pro Leu Gln	
250 255 260	
aag aag ctt cag gac ttg ctg aat gga aaa aaa tat ttg ctt gtc tta	1166
Lys Lys Leu Gln Asp Leu Leu Asn Gly Lys Lys Tyr Leu Leu Val Leu	
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ttt gtt att ccg acc ctt tct tct gtc aac aaa ttg gta gtt agt ggg Phe Val Ile Pro Thr Leu Ser Ser Val Asn Lys Leu Val Val Ser Gly 840 845 850	2894
gaa gag tca gat gca ata ggc ttc agt tcc ata tct aat ctc agg gct Glu Glu Ser Asp Ala Ile Gly Phe Ser Ser Ile Ser Asn Leu Arg Ala 855 860 865	2942
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Leu Thr Ser Leu Asn Ile Ser Tyr Asn Ser Glu Ala Thr Ser Leu Pro	
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gaa gag atg ttc aaa agc ctt gca aat cta aaa tac ttg aat atc tat	3038
Glu Glu Met Phe Lys Ser Leu Ala Asn Leu Lys Tyr Leu Asn Ile Tyr	
890 895 900	
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Tyr Phe Lys Asn Leu Lys Glu Leu Pro Thr Asn Leu Ala Ser Leu Asn	
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ccc gag gaa ggt gtg aaa ggt tta act tca ctt aca caa tta tcc ata	3182
Pro Glu Glu Gly Val Lys Gly Leu Thr Ser Leu Thr Gln Leu Ser Ile	
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aca tac tgc acg atg cta caa tgt tta tcg gag gga ttg cag cac cta	3230
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Arg Cys Glu Lys Gly Ile Gly Glu Asp Trp Tyr Lys Ile Ala His Ile	
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Pro Asp Val Phe Ile Arg	
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Lys Leu Gln Ser Thr Phe Thr Thr Ile Gln Ala Val Leu Glu Asp Ala	
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Gln Lys Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys	
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Thr Glu Ala Pro Ile Arg Gln Lys Lys Asn Lys Tyr Gly Cys Tyr His
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Pro Asn Val Ile Ala Phe Arg His Lys Ile Gly Lys Arg Met Lys Lys
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Ile Met Glu Lys Leu Asp Val Ile Ala Ala Glu Arg Ile Lys Phe His
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Leu Ala Glu Arg Thr Thr Glu Arg Gln Val Ala Thr Arg Gln Thr Gly
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Ala His Leu Arg Tyr Phe Ser Leu Thr Pro Thr Glu Leu Gly Pro Gly
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Phe Val Leu Asn Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp
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Glu Ile Val Lys Ile Leu Ile Asn Ile Val Ser Asp Ala Gln Thr Leu
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Ser Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu
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Ala Gln Met Val Phe Asn Asp Gln Arg Val Ile Glu His Phe Leu Pro
210 215 220

Lys Ile Trp Ile Cys Val Ser Glu Asp Phe Asn Glu Lys Arg Leu Ile
225 230 235 240

Lys Glu Ile Val Glu Ser Ile Glu Glu Lys Ser Leu Gly Asp Met Asp
245 250 255

Leu Ala Pro Leu Gln Lys Lys Leu Gln Asp Leu Leu Asn Gly Lys Lys
260 265 270

Tyr Leu Leu Val Leu Asp Asp Ile Trp Asn Glu Asp Gln Asp Lys Trp
275 280 285

Ala Lys Leu Arg Glu Val Leu Lys Val Gly Ala Ser Gly Ala Ser Ile
290 295 300

Leu Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gln Thr Leu
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Gln Pro Tyr Glu Leu Ser Asn Leu Cys Gln Glu Asp Cys Trp Leu Leu
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Phe Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn His Asn Leu
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Val Ala Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu
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Ala Ala Lys Thr Leu Gly Gly Ile Leu Arg Phe Lys Arg Gln Glu Arg
370 375 380

Gln Trp Glu His Val Arg Asp Ser Glu Ile Trp Lys Leu Pro Gln Glu
385 390 395 400

Glu Ser Ser Ile Leu Pro Ala Leu Lys Leu Ser Tyr His His Leu Pro
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Leu Asp Leu Arg Gln Cys Phe Ser Tyr Cys Ala Val Phe Pro Lys Asp
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Thr Lys Met Glu Lys Glu Asn Leu Ile Ser Leu Trp Met Ala His Gly
435 440 445

Phe Leu Leu Ser Lys Gly Asn Leu Glu Leu Glu Asp Val Gly Asn Glu
450 455 460

Val Trp Asn Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val
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Ala Thr Ser Leu Phe Ser Ala Ser Ala Ser Ser Asn Asn Ile Arg Glu
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Ile Asn Val Lys Gly Tyr Pro His Met Met Ser Ile Gly Phe Ala Lys
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530 535 540

Arg Val Leu Asn Leu Ser Asn Leu Glu Leu Lys Gln Leu Pro Ser Ser
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Ile Gly Asp Leu Val His Leu Arg Tyr Leu Asn Leu Ser Asp Asn Asn
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Arg Ile Arg Ser Leu Pro Lys Gln Leu Cys Lys Leu Gln Asn Leu Gln
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Thr Leu Asp Leu Arg Cys Cys Tyr Arg Leu Ser Cys Leu Pro Lys Glu
595 600 605

Thr Ser Lys Leu Gly Ser Leu Arg Asn Leu Leu Leu Asp Arg Cys His
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Gly Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys
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Glu Leu Arg Asn Leu Asn Leu Tyr Gly Ser Ile Ser Ile Thr His Leu
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Glu Arg Val Lys Asn Asp Met Asp Ala Lys Glu Ala Asn Leu Ser Ser
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Lys Glu Asn Leu His Ser Leu Ser Met Ile Trp Asp Glu Asp Glu Arg
690 695 700

Pro His Arg Tyr Glu Ser Glu Asp Val Glu Val Leu Glu Ala Leu Lys
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Pro His Ser Asn Leu Thr Cys Leu Thr Ile Ile Gly Phe Arg Gly Ile
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Arg Leu Pro Asp Trp Met Asn His Ser Val Leu Lys Asn Val Val Ser
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755 760 765

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Thr	Gln	Leu	Ser	Ile	Thr	Tyr	Cys	Thr	Met	Leu	Gln	Cys	Leu	Ser	Glu				
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Pro	Thr	Leu	Ala	Lys	Arg	Cys	Glu	Lys	Gly	Ile	Gly	Glu	Asp	Trp	Tyr				
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 Ile Gln Gly Glu Leu Val Leu Leu Phe Gly Phe Glu Asn Asp Phe Arg
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 aag ctt tca agc aca ttt tct acg atc caa ctt gtg ctt gaa gat gct 201
 Lys Leu Ser Ser Thr Phe Ser Thr Ile Gln Leu Val Leu Glu Asp Ala
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 Ser Glu Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys
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 Asn Glu Ala Ala Arg Phe Asn Gln Ser Leu Leu Gly Tyr Ile His Pro
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 Lys Ile Ile Ile Phe Arg Tyr Lys Leu Gly Lys Arg Met Lys Arg Met
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 His Leu Lys Leu Cys Leu Ala Lys Tyr Leu Leu Ile Ala
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Lys Lys Leu Gln Glu Leu Leu Asn Gly Lys Arg Tyr Phe Leu Val Leu																
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tac ttg agg tct ttc ttc caa gag gtc gaa gaa tat aaa ttt ggt aat Tyr Leu Arg Ser Phe Phe Gln Glu Val Glu Glu Tyr Lys Phe Gly Asn 470 475 480	1671
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Asp Ala Lys Glu Ala Asn Leu Ser Thr Lys Gln Lys Leu Tyr Asn Leu	
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Cys Met Ser Trp Asp Ile Arg Pro Tyr Gly Tyr Glu Ser Glu Asn Asn	
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Leu Lys Leu Tyr Asn Gly Ser Ala Glu Val Glu Tyr Ile Glu Glu Asp	
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Ile Asp Ala Ala Ser Leu Ser Ser Ile Ser Lys Leu Thr Thr Leu Thr	
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Met Phe Lys Arg Leu Val Asn Leu Glu Ser Leu Ser Ile Ile Tyr Phe	
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Lys Lys Leu Arg Glu Leu Pro Ser Ser Leu Ala Ser Leu Asn Ala Leu
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 aag tgt cta aaa att cat tat tgt tac gca cta gag agt ctc ccc gaa 3015
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 920 925 930
 caa ggg atg gaa ggg tta act tca ctc acc gac tta tat gtt caa aac 3063
 Gln Gly Met Glu Gly Leu Thr Ser Leu Thr Asp Leu Tyr Val Gln Asn
 935 940 945
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 965 970 975 980
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 Lys Leu Ser Ser Thr Phe Ser Thr Ile Gln Leu Val Leu Glu Asp Ala
 35 40 45
 Ser Glu Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys
 50 55 60
 Leu Asn Phe Ala Ala Tyr Glu Val Asp Asp Ile Leu Asp Glu Cys Lys
 65 70 75 80
 Asn Glu Ala Ala Arg Phe Asn Gln Ser Leu Leu Gly Tyr Ile His Pro
 85 90 95
 Lys Ile Ile Ile Phe Arg Tyr Lys Leu Gly Lys Arg Met Lys Arg Met
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Met Glu Lys Leu Asp Ala Ile Ala Asp Glu Arg Arg Lys Phe His Leu
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 130 135 140
 His Leu Lys Leu Cys Leu Ala Lys Tyr Leu Leu Ile Ala Thr Gly Phe
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 Val Leu Ala Glu Pro Lys Val Tyr Gly Arg Asp Lys Glu Lys Asp Glu
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 Met Val Lys Ile Leu Ile Asn Ser Val Ser Asn Ala Gln Glu Leu Leu
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 Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu Ala
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 Gln Met Ile Phe Asn Asp Gln Ser Val Thr Ala His Phe Asn Leu Lys
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 Ile Trp Val Cys Val Ser Asp Asp Phe Asp Glu Lys Arg Leu Ile Lys
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 Ala Pro Leu Gln Lys Lys Leu Gln Glu Leu Leu Asn Gly Lys Arg Tyr
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 Phe Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Glu Lys Trp Ala
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 Lys Ile Lys Ala Val Leu Lys Val Gly Ala Gln Gly Ser Ser Ile Leu
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 Ala Thr Thr Arg Leu Glu Arg Val Gly Ser Ile Met Gly Thr Trp Gln
 305 310 315 320
 Pro Tyr Gln Leu Ser Ile Leu Ser Pro Glu Tyr Cys Trp Leu Leu Phe
 325 330 335
 Lys Gln Arg Ala Phe Gly His Gln Thr Glu Thr Asn Pro Ala Leu Val
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Gly Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu Ala
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Ala Lys Thr Leu Gly Gly Leu Leu Arg Phe Lys Arg Glu Glu Ser Glu
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Trp Glu His Val Lys Asp Ser Glu Ile Trp Asn Leu Pro Gln Asp Glu
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Lys Ile Glu Lys Glu Tyr Leu Ile Thr Leu Trp Met Ala His Gly Phe
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Trp Lys Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Val Glu Glu Tyr
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Lys Phe Gly Asn Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu
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Ala Thr Ser Leu Phe Ser Thr Asn Thr Arg Ser Ser Lys Ile Arg Gln
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Ile Arg Val Ala Gln Lys Asn Thr Ile Pro Ile Gly Phe Ala Glu Val
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Val Pro Ser Tyr Ser Pro Leu Ile Phe Lys Arg Phe Val Ser Leu Arg
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Val Leu Asp Met Lys Phe Ser Lys Phe Asp Gln Leu Ser Ser Ser Ile
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Gly Asp Leu Ile His Leu Arg Leu Leu Asn Leu Arg Gly Ser Ser Ile
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Arg Ser Leu Pro Lys Arg Leu Cys Lys Leu Gln Asn Leu Gln Thr Leu
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Asp Ile Ser Cys Cys Phe Ser Leu Ser Tyr Ile Pro Lys Gln Ile Ser
595 600 605

Lys Leu Ser Ser Leu Arg Asn Leu Val Phe Ser Gly Cys Gln Ile Thr
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Ser Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu Lys Thr Leu Asp
625 630 635 640

Tyr Phe Ile Val Gly Glu Arg Lys Gly Tyr Gln Leu Gly Glu Leu Arg
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Asn Leu Ser Leu His Gly Ser Leu Ser Ile Ser His Leu Glu Arg Val
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Lys Ser Glu Thr Asp Ala Lys Glu Ala Asn Leu Ser Thr Lys Gln Lys
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Ser Glu Asn Asn Leu Asp Glu Lys Val Leu Glu Ala Leu Arg Pro His
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Ser Asn Leu Lys Ser Leu Lys Leu Ile Gly Phe Arg Gly Phe His Phe
725 730 735

Pro Asn Trp Met Asn Ala Ser Val Leu Lys Asn Val Val Ser Ile Glu
740 745 750

Ile Glu Cys Glu Asn Cys Trp Arg Leu Pro Pro Phe Gly Glu Leu Pro
755 760 765

Cys Leu Glu Ser Leu Lys Leu Tyr Asn Gly Ser Ala Glu Val Glu Tyr
770 775 780

Ile Glu Glu Asp Asp Gly His Ser Thr Leu Lys Phe Pro Tyr Leu Lys
785 790 795 800

Arg Leu Ala Ile Glu Arg Phe Pro Asn Leu Lys Gly Leu Leu Arg Ser
805 810 815

Glu Gly Glu Glu Lys Phe Ser Met Leu Glu Glu Met Glu Ile Trp His
820 825 830

Cys Pro Met Phe Val Phe Pro Ala Phe Ser Ser Val Thr Lys Leu Asp

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850	855	860
Thr Thr Leu Thr Ser	Leu Ser Ile Asp His	Asn Phe Glu Ala Thr Thr
865	870	875 880
Leu Pro Glu Glu Met	Phe Lys Arg Leu Val	Asn Leu Glu Ser Leu Ser
885	890	895
Ile Ile Tyr Phe Lys Lys	Leu Arg Glu Leu Pro	Ser Ser Leu Ala Ser
900	905	910
Leu Asn Ala Leu Lys Cys	Leu Lys Ile His Tyr	Cys Tyr Ala Leu Glu
915	920	925
Ser Leu Pro Glu Gln Gly	Met Glu Gly Leu Thr	Ser Leu Thr Asp Leu
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Tyr Val Gln Asn Cys	Glu Met Leu Lys Cys	Leu Pro Glu Gly Leu Gln
945	950	955 960
His Leu Arg Ala Leu Thr	Ser Leu Gln Ile Tyr	Gly Cys Pro Ala Leu
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Cys Phe Ile Gln Gly Glu Leu Gly Leu Ile Leu Gly Phe Lys Asp Glu
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Phe Glu Lys Leu Gln Ser Thr Phe Thr Thr Ile Gln Ala Val Leu Glu
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Asp Ala Gln Lys Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu
50              55              60
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Tyr Gly Leu Thr Cys Met Pro Pro Arg Ile Gly Ser Leu Thr Cys Leu							
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Lys Thr Leu Ser Arg Phe Val Val Gly Ile Gln Lys Lys Ser Cys Gln							
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His Leu Glu Arg Val Lys Asn Asp Met Asp Ala Lys Glu Ala Asn Leu							
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Ser Ala Lys Glu Asn Leu His Ser Leu Ser Met Lys Trp Asp Asp Asp							
	675 680 685						
gaa cgt cca cgt ata tat gaa tca gaa aaa gtt gaa gtg ctt gaa gct	3549						
Glu Arg Pro Arg Ile Tyr Glu Ser Glu Lys Val Glu Val Leu Glu Ala							
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Leu Lys Pro His Ser Asn Leu Thr Cys Leu Thr Ile Arg Gly Phe Arg							
	705 710 715						
gga atc cgt ctc cca gac tgg atg aat cac tca gtt ttg aaa aat gtt	3645						
Gly Ile Arg Leu Pro Asp Trp Met Asn His Ser Val Leu Lys Asn Val							
	720 725 730 735						

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835 840 845	
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865 870 875	
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 Trp Tyr Lys Ile Ala His Ile Pro Arg Val Phe Ile Tyr
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Lys Leu Gln Ser Thr Phe Thr Thr Ile Gln Ala Val Leu Glu Asp Ala
 35 40 45

Gln Lys Lys Gln Leu Lys Asp Lys Ala Ile Glu Asn Trp Leu Gln Lys
 50 55 60

Leu Asn Ala Ala Ala Tyr Glu Ala Asp Asp Ile Leu Asp Glu Cys Lys
 65 70 75 80

Thr Glu Ala Pro Ile Arg Gln Lys Lys Asn Lys Tyr Gly Cys Tyr His
 85 90 95

Pro Asn Val Ile Thr Phe Arg His Lys Ile Gly Lys Arg Met Lys Lys
 100 105 110

Ile Met Glu Lys Leu Asp Val Ile Ala Ala Glu Arg Ile Lys Phe His
 115 120 125

Leu Asp Glu Arg Thr Ile Glu Arg Gln Val Ala Thr Arg Gln Thr Gly
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Phe Val Leu Asn Glu Pro Gln Val Tyr Gly Arg Asp Lys Glu Lys Asp
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Glu Ile Val Lys Ile Leu Ile Asn Asn Val Ser Asn Ala Gln Thr Leu
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Pro Val Leu Pro Ile Leu Gly Met Gly Gly Leu Gly Lys Thr Thr Leu
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Ala Gln Met Val Phe Asn Asp Gln Arg Val Ile Glu His Phe His Pro
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Lys Ile Trp Ile Cys Val Ser Glu Asp Phe Asn Glu Lys Arg Leu Ile
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Lys Glu Ile Val Glu Ser Ile Glu Glu Lys Ser Leu Gly Gly Met Asp
 225 230 235 240

Leu Ala Pro Leu Gln Lys Lys Leu Arg Asp Leu Leu Asn Gly Lys Lys
 245 250 255

Tyr Leu Leu Val Leu Asp Asp Val Trp Asn Glu Asp Gln Asp Lys Trp
 260 265 270

Ala Lys Leu Arg Gln Val Leu Lys Val Gly Ala Ser Gly Ala Ser Val
 275 280 285

Leu Thr Thr Thr Arg Leu Glu Lys Val Gly Ser Ile Met Gly Thr Leu
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Gln Pro Tyr Glu Leu Ser Asn Leu Ser Gln Glu Asp Cys Trp Leu Leu
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Phe Met Gln Arg Ala Phe Gly His Gln Glu Glu Ile Asn Leu Asn Leu
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Val Ala Ile Gly Lys Glu Ile Val Lys Lys Cys Gly Gly Val Pro Leu
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Ala Ala Lys Thr Leu Gly Gly Ile Leu Arg Phe Lys Arg Glu Glu Arg
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 Gln Trp Glu His Val Arg Asp Ser Glu Ile Trp Lys Leu Pro Gln Glu
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 Glu Ser Ser Ile Leu Pro Ala Leu Arg Leu Ser Tyr His His Leu Pro
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 Leu Asp Leu Arg Gln Cys Phe Thr Tyr Cys Ala Val Phe Pro Lys Asp
 405 410 415
 Thr Glu Met Glu Lys Gly Asn Leu Ile Ser Leu Trp Met Ala His Gly
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 Phe Ile Leu Ser Lys Gly Asn Leu Glu Leu Glu Asn Val Gly Asn Glu
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 Val Trp Asn Glu Leu Tyr Leu Arg Ser Phe Phe Gln Glu Ile Glu Val
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 Lys Ser Gly Gln Thr Tyr Phe Lys Met His Asp Leu Ile His Asp Leu
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 Ala Thr Ser Leu Phe Ser Ala Ser Thr Ser Ser Ser Asn Ile Arg Glu
 485 490 495
 Ile Ile Val Glu Asn Tyr Ile His Met Met Ser Ile Gly Phe Thr Lys
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 Val Val Ser Ser Tyr Ser Leu Ser His Leu Gln Lys Phe Val Ser Leu
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 Arg Val Leu Asn Leu Ser Asp Ile Lys Leu Lys Gln Leu Pro Ser Ser
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 Ile Gly Asp Leu Val His Leu Arg Tyr Leu Asn Leu Ser Gly Asn Thr
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 Ser Ile Arg Ser Leu Pro Asn Gln Leu Cys Lys Leu Gln Asn Leu Gln
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Gly Glu Leu Arg Asn Leu	Asn Leu Tyr Gly Ser	Ile Glu Ile Thr His
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Leu Glu Arg Val Lys Asn	Asp Met Asp Ala Lys	Glu Ala Asn Leu Ser
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Ala Lys Glu Asn Leu His	Ser Leu Ser Met Lys	Trp Asp Asp Asp Glu
675	680	685
Arg Pro Arg Ile Tyr Glu	Ser Glu Lys Val Glu	Val Leu Glu Ala Leu
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Lys Pro His Ser Asn Leu	Thr Cys Leu Thr Ile	Arg Gly Phe Arg Gly
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Ile Arg Leu Pro Asp Trp	Met Asn His Ser Val	Leu Lys Asn Val Val
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Gly Glu Leu Pro Cys Leu	Lys Ser Leu Glu Leu	Trp Arg Gly Ser Ala
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Glu Val Glu Tyr Val Asp	Ser Gly Phe Pro Thr	Arg Arg Arg Phe Pro
770	775	780
Ser Leu Arg Lys Leu Asn	Ile Arg Glu Phe Asp	Asn Leu Lys Gly Leu
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Leu Lys Lys Glu Gly Glu	Glu Gln Cys Pro Val	Leu Glu Glu Ile Glu
805	810	815
Ile Lys Cys Cys Pro Met	Phe Val Ile Pro Thr	Leu Ser Ser Val Lys
820	825	830
Lys Leu Val Val Ser Gly	Asp Lys Ser Asp Ala	Ile Gly Phe Ser Ser
835	840	845

Ile Ser Asn Leu Met Ala Leu Thr Ser Leu Gln Ile Arg Tyr Asn Lys
 850 855 860
 Glu Asp Ala Ser Leu Pro Glu Glu Met Phe Lys Ser Leu Ala Asn Leu
 865 870 875 880
 Lys Tyr Leu Asn Ile Ser Phe Tyr Phe Asn Leu Lys Glu Leu Pro Thr
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